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Environmental Risk Assessments and the Regulation of Genetically Engineered Plants in the United States

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It is a very great honor and pleasure to be able to share some thoughts with you during this symposium about how we evaluate the environmental safety of transgenic plants.

I would like to thank the organizers for providing this opportunity for all of us to hear a variety of perspectives, including those of research scientists and government regulatory scientists. As we have often seen over the years, the free exchange of ideas makes it possible to have a incorporate the best available scientific knowledge when using risk assessments (RAs) to make decisions into a dynamic regulatory system.

In the interest of clarity, let me to note at the outset that I will tend to use the term "genetically engineered" or GE rather than the term "transgenic." This field seems to have a proliferation of terms that are often used synonymously, such as recombinant, transgenic, GE, GM, GMO, and LMO. My use of the term GE is not intended to minimize any preference that some may have for some of these other terms.

For today's presentation, I would like to discuss each of the following:

1. The US regulatory system and when the regulatory agencies use environmental RA to inform the decision-making process
2. Main elements of the RA methodology
3. Examples of using the RA methodology in specific cases
4. RA and decision-making
5. Conclusions

1. The US regulatory system and when the agencies use environmental RA

Let me set the stage with a brief sketch of the regulatory structure in the United States that applies for transgenic plants and other products of biotechnology. As you may know, in the United States we did not pass a separate biotechnology law to regulate GE organisms, but instead operate under a Federal Coordinated Framework for the Regulation of Biotechnology. We have used the existing laws for comparable traditional organisms and products, and then applied these laws in each specific case of a GE organism.

Generally, three agencies are responsible for the regulation of GE plants and other GE organisms:

- The U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) is a regulatory agency responsible for the protection of agriculture and the environment against pests and diseases. Within APHIS, Biotechnology Regulatory Services (BRS) is responsible for implementing the APHIS biotechnology regulations that govern the importation, interstate movement, and environmental release of GE plants

and other GE organisms that may pose a risk of possible direct or indirect harm to plants.

- The Environmental Protection Agency (EPA) is responsible for the assuring the safe use of pesticidal substances, whether applied externally, like traditional herbicides and insecticides, or incorporated into the plant itself in the case of GE plants engineered to produce a pesticidal substance within the plant's tissues. EPA also sets the allowable levels, or "tolerances", of a pesticide that can be on or in food.

- The Food and Drug Administration (FDA) is responsible for assuring the safety of the U.S. food, drugs, and cosmetics. Under FDA's authority, animal feed falls under the heading of food.

Each of the agencies has highly trained staff members assigned to conduct safety reviews that will be used by decision makers in our agencies.

In light of the symposium's focus on environmental risk assessments, we won't hear more about FDA reviews, but those who are interested may want to look at the considerable information that FDA provides online about their regulatory approach (http://www.cfsan.fda.gov/~lrd/biotechm.html).

APHIS and EPA environmental RAs come into play at certain stages of research and development of GE plants:

- When permits are issued for field tests (controlled, environmental releases)
  - APHIS regulates all GE plants

- EPA regulates GE plants that produce a plant incorporated protectant or PIP (e.g., the Bacillus thurinigiensis protein)

∞ Just prior to commercialization.

- APHIS reviews prior to granting nonregulated status
- EPA reviews prior to registering the PIP
- EPA reviews to set tolerance levels for PIP in food and feed (allowable, safe levels)

∞ In response to compliance infractions, such an unauthorized environmental release, the RA can be used to decide on the need for remedial actions.

2. Main elements of the methodology for RA

RA was developed as a formal discipline during the latter half of the 20th century to address questions related to chemical and radiation safety. Many of these RAs used quantitative approaches, although qualitative RAs have been used as well. The main elements of the RA are relatively simple, and they can be applied flexibly to address a wide range of situations. They are sometimes expressed simply as risk being a function of a hazard and the likelihood of exposure to the hazard.

Hazard x Exposure = Risk

When assessing the risk of a simple system, the RA can be fairly simple, but GE plants are usually not a simple system. Scientists and government regulators around the world have worked together to identify useful information and approaches that can be used in a practical way for conducting RA of GE plants.

Even before the creation of the first GE plant in 1983, scientists and regulators around the world were discussing the best ways to approach RA for these and other GE organisms. A great deal of international collaboration was done by countries working under the umbrella of the Organization for Economic Co-operation and Development (OECD). The first OECD report contained a number of recommendations, including that for the public to have confidence in the products of modern biotechnology, governments must have proper mechanisms in place to regulate their safety ("International Trends and Perspectives", OECD 1982).

In 1986, OECD published the first guidance document entitled "Recombinant DNA: Safety Considerations" (sometimes referred to as the 1986 OECD 'Blue Book'). This was the first OECD publication to respond to the 1982 recommendation for safe regulation. It put forward key safety concepts for development and commercialization of GE organisms, including GE plants. The Blue Book included advice on several topics including risk assessment, agriculture and the environment, and concepts for safely conducting small-scale field testing of GE plants. The advice in the Blue Book was developed with input from hundreds of experts from OECD countries, and has since been used as the basis of regulation of GE plants in many countries.

The Blue Book was followed by subsequent guidance documents echoed many of the same considerations that could be taken into account when evaluating the environmental safety of GE organisms. Other similar and complementary guidance was included in the UNEP International Technical Guidelines for Safety in Biotechnology (1995) and Annex III of the Cartagena Protocol on Biosafety (2000).

Representatives of the U.S. regulatory agencies were active participants in the international discussions that led to the development of these guides.

The United States has also worked closely with other countries to assemble information useful in the evaluation of GE plants, including information related to traditional agricultural practices. Two examples of this include the 1993 OECD work on "Traditional Crop Breeding Practices, A Historical Review to Serve as a Baseline for Assessing the Role of Modern Biotechnology" and the numerous plant species consensus documents on developed cooperatively by member countries working under the auspices of the OECD's Working Group on Harmonisation of Regulatory Oversight in Biotechnology. For access to Consensus Documents for the Work on Harmonisation of Regulatory Oversight in Biotechnology, see the website links available at http://www.oecd.org/document/51/0,2340,en_2649_34385_1889395_1_1_1_1,00.html.

These OECD documents highlight the widespread acceptance of a comparative approach when evaluating the safety of GE plants. This comparative approach is used not only in the United States, but also in many other countries around the world. This approach takes into account the great deal of prior experience we have accumulated in evaluating the traditional plants.

This is an outgrowth of the concept of familiarity, which was described in the 1993 OECD document on "Scale-up" (Safety Considerations for Biotechnology: Scale-up of Crop Plants, OECD, 1993). The familiarity concept "...is based on the fact that most genetically engineered organisms are developed from organisms such as crop plants whose biology is well understood". "Familiarity allows the risk assessor to draw on previous knowledge and experience with the introduction of plants and

micro-organisms into the environment..." For plants, familiarity takes account of a wide-range of attributes including, for example, knowledge and experience with "the crop plant, including its flowering/reproductive characteristics, ecological requirements, and past breeding experiences". This provides risk assessors with a useful tool in reviewing information related to the biology of the host organism when conducting a RA.

The concept of familiarity is based on the fact that most GE organisms are developed from organisms such as crop plants whose biology is well understood. Familiarity is not a RA in itself, but the concept facilitates RA. Familiarity often means having enough information to be able to make a judgment of safety or risk (U.S. National Academy of Sciences, 1989). Familiarity can also be used to indicate appropriate management practices including whether standard agricultural practices are adequate or whether other management practices are needed to manage the risk. Familiarity allows the risk assessor to draw on previous knowledge and experience with the introduction of non-GE plants and micro-organisms into the environment, and this prior experience can indicate appropriate management practices for introductions of the GE-counterparts. As familiarity depends also on the knowledge about the environment and its interactions with introduced organisms, in some cases the RA in one country may not be applicable in another country. However, as more field tests are performed, it should be possible to derive additional information about the organisms involved, and their interactions with a number of environments.

Recognizing that there is a general agreement of the general approaches used when conducting environmental RA for GE plants and other GE organisms, a group of public sector researchers have developed a useful, step-wise guide for conducting an

environmental RA. This approach is summarized below, and has the advantage of being consistent with the approach used in the United States and most other countries that are conducting environmental RA of GE organisms. Further information on the guide developed under this initiative can be found at their website (http://pubresreg.org/). The guide is currently in its second draft, and the steering committee is accepting comments and suggestions before making revisions (see "Draft guide for Notifications and Risk Assessments for Releases of GMOs" online at http://pubresreg.org/Members/Kim/working%20groups/biosafety%20protocol/CPB/Notificationguide).

The methodology for RA typically follows the following steps:

- Hazard identification
- Likelihood estimation
- Consequence evaluation, including a baseline assessment
- Risk estimation
- Risk management
- Consideration of overall risk

A phased approach provides a systematic way of working through the RA:

Phase 1: Consideration of each of the inserted genes and sequences individually.

Phase 2: Consideration of the whole plant, including available empirical information on the resulting GMO in order to evaluate potential unintended effects or changes to the GE plant (e.g., synergistic effects, insertion effects).

Phase 3: Consideration of risk management and overall risk.

In the case of the first two phases of the RA, looking at the inserted genes then the whole plant, the risk assessor works through the first three steps of hazard identification, likelihood estimation, then considering the consequences (includes a consideration of a baseline assessment which is often a comparison with the non-GE counterpart of the GE plant).

Looking at the hazard identification, there are a several key considerations, especially considering that this will drive the other steps of the RA. Throughout the identification of potential hazards, it is important that there are plausible, scientifically sound reasons for suggesting that something may pose a hazard. Another key consideration is that the "hazard" is actually a hazard and not just something that occurs. Many risk assessors find that potential hazards are more easily clarified if the assessor writes down a clear description of the potential hazard. This makes it easier to see when the "hazard" is too vague to be readily evaluated. Clarity at this step makes it easier to identify which information in really relevant in not only ascertaining the nature of the hazard, but also estimating the likelihood of the hazard occurring and the resultant consequences. This written description of the potential hazard also makes it easier for the risk assessor to get additional input from people who may have greater knowledge necessary for the comparison to the non-GE plant or the existing agricultural practices.

Consideration of the inserted genes and sequences is often fairly straightforward if the risk assessor has firsthand knowledge or access to the scientific literature. Oftentimes, it is more difficult to decide how to evaluate the whole plant, i.e., all of the potential changes that may have occurred apart from the expected phenotype of the introduced genes and sequences.

There has been considerable interest in finding useful ways to evaluate the possible unintended changes that may have occurred in the GE plant. It is well known from non-GE plants that plants exhibit heritable, unintended genetic changes that may be caused by spontaneous mutations arising from chemicals, radiation, or other environmental factors. The pioneering work of Barbara McClintock on maize transposable elements has led to the discovery of similar transposable elements in virtually every plant species where researchers have sought them out, so it is clear that genetic changes may be mediated by endogenous transposable elements. Soma-clonal variation is another mechanism that has been frequently found in plants that have undergone tissue culture, as many GE plants have (as well as many non-GE plants). These are just a few examples that make clear the need to be able to address the potential for unintended changes to the GE plant, but how is this done in a practical manner?

Two ideas mentioned previously, the concept of familiarity and the comparative approach, have pointed the way for risk assessors to gather information that can be used to evaluate the likelihood, or unlikelihood, that there have been unintended changes made to the GE plant. Over the course of several years of technical discussions related to RAs done by USDA-APHIS and their counterparts in the Canadian Food Inspection Agency (CFIA) found that they used a similar approach that was useful in evaluating whether it was likely that unintended changes had occurred in a GE plant. These discussions arose from reviews that each agency had done when developers sought the final approvals before commercialization of GE plants. The risk assessors decided to develop a document that would be describe their experience in their RAs to date, and thereby more clearly describe the approach they had found useful in addressing these questions. (APHIS and CFIA refer to these appendices as “living documents” to be

periodically updated to reflect experience accumulated over time; for more information see http://www.inspection.gc.ca/english/plaveg/bio/harmone.shtml).

This "Appendix 2" describes the type of environmental information that is most commonly seen in the applications to CFIA and APHIS just prior to commercialization. One section of "Appendix 2" deals with "Phenotype of the transgenic plant" and lists a number of characteristics that may be useful in making inferences about whether unintended changes were likely or unlikely to have occurred in the GE plant. These are the characteristics of plants that we have a great deal of familiarity in evaluating, since they are the characteristics that are of interest to plant breeders, agronomists and horticulturalists. By looking at the information about the GE plant's phenotypic characteristics, it is possible to draw inferences about whether it is likely or unlikely that unintended changes have occurred to the GE plant. Each of these phenotypic characteristics is controlled by many genes, and a lack of change in the phenotype can lend support to the conclusion that these many genes have not been perturbed. This is the logic behind this approach. There is also good empirical evidence to support this approach, because this is the approach that humans have used for thousands of years in improving various plant species.

Information on the plant's phenotypic characteristics is also a way to provide supporting evidence that the plant is unlikely to pose a problem in being more invasive or environmentally persistent than the non-GE counterpart of the plant (i.e., be more of a problem as a weed). The list in the Appendix 2 section on "phenotype" suggests possible parameters to measure for the GE plant. The list is not mandatory or exclusive of other possible characteristics, but gives guidance on practical parameters with which plant scientists have a great deal of experience. Examples

include: growth habit, life-span, vegetative vigor, ability to overwinter or overseason), number of days to onset of flowering; number of days for flowering, number of days until maturity, seed parameters (e.g., seed, dormancy, seedling emergence), proportion of plants surviving from seedling to reproduction, outcrossing frequency, interactions with pollinator species, pollen parameters (physical, biochemical, biological), fertility, asexual reproduction, seed dispersal factors, interaction with symbionts (e.g., vesicular-arbuscular mycorrhizal fungi, rhizobia), and stress adaptations (e.g., to biotic or abiotic stressors).

This is just a brief overview of Appendix 2 and how it can be useful in conducting an environmental RA. The full text can be found online at http://www.inspection.gc.ca/english/plaveg/bio/usda/appenannex2e.shtml.

3. Examples of using the RA methodology in specific cases

Confined environmental releases – field tests

In the case of field tests, these are controlled environmental releases that are limited in geographic area and in duration. In a typical test, the GE plant is in the environment for several months, starting with planting and ending with harvest. In the season following harvest, the test site is monitored to detect any volunteer plants that may have survived from the test, and these plants are eradicated before they can disseminate and persist in the environment. Because the opportunities for environmental interaction are relatively limited in field testing, the key consideration for reducing any possible a risk is to confine the GE plants during the test and afterward. If we were to relate this to the RA equation from above, the confinement serves to reduce the exposure or likelihood parameter.

Decisions on confinement approaches take into account our familiarity with the plant biology, especially the reproductive biology. Confinement decisions also take into account the phenotype of the GE plant and whether the phenotype is similar in kind to the phenotypes that of non-GE plants. Many, if not most, GE plants field tested to date have been developed to express characteristics similar to the characteristics that have been bred into traditional plant varieties. These characteristics include resistance to pathogens and insect pests, improved quality traits, increased yield, and even tolerance to certain herbicides. Appropriate confinement approaches, then, take into account the gene(s) introduced, the resultant phenotype, and the similarity of the phenotype to plants with which we have experience from traditional plant improvement. Likewise, the confinement approach considers the experience gained in the production of specific plant phenotypes in the course of plant breeding practices and production of certified seed varieties.

Historically in the United States and elsewhere, the isolation distances for certified seed production have been commonly used as part of the confinement practices used for field testing GE plants that have been engineered with traits that are similar in kind to the varieties commonly developed through traditional plant breeding programs. These are the types of GE plants that have been most frequently field tested in the United States and other countries.

In recent years, there has been some limited field testing of GE plants that have been engineered to express traits that are not similar to the traits used in traditional plant variety improvement programs. In many instances, the goal is not to produce a new variety to go into general agricultural production, but rather to use the GE plants to produce a high-value protein that can be extracted from the plants after the test.

After extraction, the protein would be further processed or formulated into a product (e.g., medicinal or therapeutic compounds, food processing agents, enzymes for use in manufacturing processes).

Because these types of GE plants are so dissimilar from the plants we are most familiar with, APHIS and regulatory officials in other countries have chosen to use more stringent approaches to achieving high levels of confinement of these GE plants when grown in the controlled environmental releases (in many ways the term field test does not apply as well, since that term is more associated with typical agronomic field tests done for variety development). As one might expect, the rigor for these environmental releases takes considerable preparation, long before an application is submitted to APHIS for a field test. APHIS has provided copious guidance to prospective applicants, and the agency has recently reformatted this guidance in a consolidated User's Guide that is available from the website of APHIS' Biotechnology Regulatory Services (http://www.aphis.usda.gov/brs/pdf/Pharma_Guidance.pdf).

Unconfined environmental releases – determination of nonregulated status

Let me turn briefly to the environmental RA that APHIS does when someone petitions for the agency to grant a determination of nonregulated status. This would be analogous to the reviews done in some countries when developers seek authorization for placing on the market. In the course of the assessment APHIS considers the range of potential impacts on the environment arising from a decision to grant nonregulated status. With nonregulated status the GE plant is no longer subject to APHIS authorizations when importing, moving interstate, or releasing the GE plant into the environment. These are very extensive reviews, and encompass more information than needed for a RA prior to authorizing a field test.

When we discussed RA for field tests, we said that confinement of the GE during the controlled release was a key part of the evaluation. In the case of the RA when a developer petitions APHIS for nonregulated status for a GE plant, confinement doesn't have the same significance, since the nonregulated GE plant will be unconfined and used in the same manner as other non-GE plants. Therefore, the RA does not focus on ascertaining outcrossing rates, but rather focuses on determining what the biological consequence is likely to be if and when outcrossing occurs.

Let me share an example from several years ago when APHIS reviewed a petition to grant nonregulated status for rice lines that had been engineered to express phosphinothricin acetyltransferase (PAT), the enzyme that confers tolerance to the broad-spectrum herbicide glufosinate. There was some initial concern, because in the United States red rice is a close relative of cultivated rice, and red rice is a problem weed in rice production. Control of red rice is difficult in part because the herbicides that will kill red rice will also kill the rice crop. In light of this, the developer wanted to develop a cultivated rice variety that would be able to tolerate exposure to a broad spectrum herbicide like glufosinate, and thereby give the farmer an additional way to control red rice weeds in the rice fields.

Outcrossing of rice to red rice does occur, although at a very low frequency, but in this RA, we focused more on the consequences of the outcrossing rather than trying to make precise determinations of the outcrossing frequency. In doing so, we recognized that there were adequate management tools available to prevent any outcrossing from having a significant impact on the options available to growers or the environment itself. In essence, all of the existing weed control strategies then in

use for red rice in rice production would still be effective in controlling any red rice plants that might acquire the *pat* gene and glufosinate tolerance from the GE rice.

This petition review highlighted another important lesson when conducting an environmental RA. It's important for the reviewers to write down as much of the steps and thought process as possible and thereby lay out each scenario is detail. The detailed scenario helps to clarify which information is needed and which information is peripheral or not needed at all to address the potential hazard that has been identified during the steps of the RA.

In the course of conducting this RA, our reviewers had some very interesting discussions around a term that many of us have heard over the years. The term is "super weed", and it is one that seems to have captured the imagination of the popular press and some members of the general public. We heard fears that if the *pat* gene outcrossed from the GE rice to the red rice, the red rice would become a "super weed". We addressed this issue in our RA and reached the conclusion that if red rice did acquire the pat gene, the red rice would no longer be sensitive to the herbicide glufosinate. This would not affect its sensitivity to other herbicides, whether broad spectrum like glyphosate or narrow spectrum like the graminicides. Other weed control mechanisms, such as flooding, tillage, and crop rotation, would still be just as effective in controlling the red rice that had acquired the tolerance to glufosinate. So the myth of super weed is not warranted. In fact, our colleagues in weed science find this public concern about super weeds to be a notion that has more support from public perception than from scientific fact.

This example makes clear, once again, how important it is to not only work through the RA in a methodical, systematic manner, but also to clearly summarize how the RA was done and how the conclusions were reached. Sometimes scientists do not realize that the logic of their assessment is not clear if the reader does not have the same background to fill in the details or connections. In general, it is always possible to improve the clarity of the summaries of our RAs. This builds the confidence of the decision-makers, as well as the general public. This clarity is also beneficial for future petitioners, so they can better understand the nature of the RAs that are done.

In the United States, guidance to developers is an important ingredient in making a regulatory system work more smoothly. Each of the U.S. regulatory agencies provides guidance to developers that are relevant to different stages in the research and development of GE plants. In addition to written guidance (accessible through agency web sites) each of the agencies encourages early and frequent consultations to provide further information. The web sites also provide access to relevant resource materials, including materials that serve as frequent references when conducting RAs.

CONCLUSIONS

In this brief introduction, I hope I have provided a flavor of our experience in the United States over the years in conducting environmental RA on the use of GE plants. Our approaches have been developed and refined over many years in which we have continued to seek out and use the best available scientific information when doing our environmental RAs.

Although I have touched only briefly on the importance of clear documents to summarize the RAs to both technical experts and the general public, it is important to

mention that these documents make it possible to build and maintain the confidence of others in the quality and conclusions of our RAs. Likewise, these documents become useful in the bilateral and multilateral technical discussions we have with our counterparts in other countries who are engaged in assessing the environmental consequences of using GE plants.

SELECTED RESOURCES FOR ADDITIONAL INFORMATION

United States Regulatory Agencies Unified Biotechnology Web page for information on US regulation of GE organisms and role of US regulatory agencies: http://usbiotechreg.nbii.gov/

Recombinant DNA Safety Considerations. Safety considerations for industrial, agricultural and environmental applications of organisms derived by recombinant DNA techniques ("The Blue Book"), OECD, 1986.

Field Testing of Genetically Modified Organisms: Framework for Decisions. U.S. NAS - National Academy of Sciences, National Academy Press, Washington DC. USA 1989.

Safety Considerations for Biotechnology: Scale-up of Crop Plants, OECD. 1993a.

Traditional Crop Breeding Practices: A Historical Review to serve as a Baseline for Assessing the Role of Modern Biotechnology, OECD 1993b.

Commercialization of Agricultural Products Derived through Modern Biotechnology: Survey Results, OECD 1995a.

Report of the OECD Workshop on the Commercialization of Agricultural Products Derived through Modern Biotechnology, OECD 1995b.

Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology, OECD 1995c.

"Draft guide for Notifications and Risk Assessments for Releases of GMOs" http://pubresreg.org/Members/Kim/working%20groups/biosafety%20protocol/CPB/Notificationguide